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Accurate Needle-Free Assessment of Myocardial Oxygenation for Ischemic Heart Disease

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ABSTRACT

The heart falls in love and helps with decisions, but it couldn't do either without sufficient oxygen, and on demand. The capability of blood vessels to supply the heart muscle (myocardium) with that oxygen, called myocardial oxygenation, is the critical determinant of cardiac function. Impairment of myocardial oxygenation is a defining feature of ischemic heart disease (IHD), which afflicts millions of people around the world. IHD is caused by pathological conditions that affect the blood vessels supplying oxygen to the heart muscle. Methods for detecting myocardial oxygenation deviations from the norm are routinely sought to guide interventions (medical, surgical or lifestyle) and to prevent acute life-threatening events such as heart attacks (myocardial infarction). Despite their importance, efforts to attain noninvasive methods for ascertaining myocardial oxygenation have not yet been realized. Instead, the current diagnosis of IHD still relies on surrogate metrics, which are either non-specific, operator dependent, or require ionizing radiation or contrast agents (injected dyes): many patients are accordingly contraindicated. Two decades ago, an oxygenation-sensitive CMR approach was used to demonstrate that CMR signals can be sensitized to changes in myocardial oxygenation. But physiological and imaging noise during CMR data acquisition persistently limited the ability to detect small changes in oxygenation-sensitive CMR signals in the heart, impeding its adoption. Here, we demonstrate a CMR-based approach (cfMRI denoting cardiac functional MRI) that jumps the barrier, finally allowing reliable detection of myocardial oxygenation. It does this by (a) advancing a natural molecule (carbon dioxide), for repeat interrogation of the functional capacity of the heart's blood vessels; (b) developing a fast MRI approach suitable for clinical adoption, to efficiently gather time-resolved oxygenation-sensitive signals throughout the heart, without being limited by key confounders (cardiac/respiratory motion and heart-rate changes, etc.); and (c) integrating the multiple whole-heart images within a computational framework, to statistically reduce noise and arrive at confidence maps of alterations in myocardial oxygenation. At a minimum, cfMRI enables evaluation of IHD for those currently contraindicated for current state-of-the-art imaging: it does not require ionizing radiation, contrast agents or needles (venous cannulations). It may even prove beneficial for general application, as cost, side effects, and risk of adverse events may be less. At a maximum, cfMRI permits monitoring of myocardial oxygenation, which opens the potential to observe previously unattainable image data to enhance our current understanding of cardiac physiology and pathophysiology. This has the potential to broaden our ability to noninvasively identify those at risk for IHD and a diverse spectrum of heart diseases related to myocardial ischemia.

INTRODUCTION

Ischemic heart disease (IHD) is the leading cause of death in the Western world (1). It often stems from atherosclerotic narrowing of the coronary arteries (stenosis), leading to reduced blood flow and oxygen supplied to the heart muscle (myocardium). This causes myocardial ischemia during physical exertion, a condition where the oxygen supply to the heart muscle does not meet the myocardial oxygen demand (2). The presence and extent of myocardial ischemia are key predictors of major adverse cardiac events (MACE), including stroke, heart attack (myocardial infarction) and death (1). Early interventions (medical, surgical or lifestyle), guided by the extent and severity of ischemia, are crucial for reducing MACE in IHD patients (3-5). Yet, to date, there are no reliable noninvasive methods to evaluate the presence or severity of deficiencies in meeting myocardial oxygen demands.

Given the lack of viable methods to assess myocardial oxygenation, the diagnosis of IHD has become entrenched in the use of a surrogate metrics, notably electrocardiography (ECG) or myocardial blood flow (MBF). Among these, the determination of ongoing myocardial ischemia based on ECG changes is attractive since ECG assessment is highly accessible. Yet, ECG may be non-specific, can be normal in patients during an ischemic event and cannot identify asymptomatic patients with significant coronary stenosis unless combined with exercise stress, which is not tolerated by more than 50% of IHD patients (6). Methods based on MBF are the most widely used for the assessment of IHD and one that is recommended by the American Heart Association. MBF changes can be determined using multitudes of clinically available imaging methods, including single-photon emission tomography (SPECT), positron-emission tomography (PET), first-pass perfusion cardiac magnetic resonance imaging (MRI) and contrast-enhanced echocardiography (7). These methods are often combined with exercise stress or intravenously injected stress agent (e.g., adenosine), to assess ischemic burden (extent and severity). In their use, these methods suffer from one or more problems that limit diagnostic capabilities and, ultimately, patient outcomes. For example, SPECT and PET approaches are used in more than 90% of the nearly 10 million myocardial ischemia-testing studies in the US (1), but they require radioactive tracers, which pose incremental risk to patients (8). Other methods, such as first-pass perfusion MRI, are free of ionizing radiation but require intravenously delivered exogenous contrast media, based on gadolinium (7), which are contraindicated in millions of people in the US with chronic kidney disease (9). More recently, a T1-based MRI method that does not require exogenous contrast agents or ionizing radiation, likely based on impairments in myocardial blood volume, has been demonstrated in IHD patients (10). However, because oxygen supply and demand to a given physiological stimulus are variable in every patient, measuring MBF may not provide full physiological insight into the extent and severity of myocardial ischemia in patients with IHD (11). Further, there are pathological conditions where perfusion is normal but oxygenation is impaired (12) (13). For all of these reasons, the quest for development of a non-invasive method has been actively pursued by researchers.

To assess IHD based on myocardial oxygenation without ionizing radiation or exogenous contrast media, blood-oxygen-level-dependent (BOLD) cardiac MRI (BOLD-CMR) has been investigated (14). The evidence that BOLD-CMR changes are primarily based on myocardial oxygenation was presented nearly two decades ago (15, 16). This led to several pilot clinical validation studies, testing the method's feasibility (12, 17, 18). However, the state-of-the-art BOLD-CMR approach has been shown to perform poorly against the current "state of the art" PET (12). It is not known whether this discrepancy is real (i.e., a true one between a test for myocardial oxygenation and a test for myocardial blood flow) or

whether it is a consequence of known accuracy limitations of BOLD-CMR. Without a reliable approach to assess myocardial oxygenation, there is no way to directly evaluate the method's status in IHD, its relationship to myocardial blood flow, and its potential for diagnosis for new, "at-risk" patients, where impaired oxygenation does not accompany detectable abnormalities in blood flow.

Uncertainty of a measurement is fundamentally determined by noise. In BOLD-MRI, physiological noise (motion) and imaging noise (limitations in signal-reception elements, termed "RF coils") dominate the small signal changes that result from oxygenation level changes. This makes it challenging to accurately index an observed signal change against blood oxygenation. The realization that the uncertainty of a measured signal can be reduced if the response can be repeatedly modulated by a known stimulus (19), was a major breakthrough for BOLD-MRI in the brain (functional MRI, abbreviated as fMRI) and it enabled sensitivity required for accurate detection of oxygenation changes. Notably, this key innovation has enabled fMRI to revolutionize our understanding of neural processes over the past two decades (20). Though this approach for defeating the critical noise limitations of BOLD-CMR is appealing, translating it into practice for myocardial oxygenation assessment is far from simple. Unlike in the brain, where the vasoactive stimulus is easily repeatable because it is visual or cognitive, injectable drugs (e.g., adenosine) are used to stimulate changes in the heart and these cannot be repeatedly administered within the same examination due to adverse side effects (21). To statistically uncover the underlying BOLD-CMR signals in the heart, rapidly acquired images registered across multiple stimulations, as well as whole-heart BOLD-CMR images are required. However, state-of-the art BOLD-CMR is essentially two-dimensional: it is typically limited to a single slice, because whole-heart three-dimensional acquisitions that are sensitive to oxygenation cannot be completed within the time that pharmacological agents are administered. The current acquisition schemes are also sensitive to heart-rate variations between different vasodilatory states. This means they can contaminate the BOLD-CMR signal readouts by masking the signal associated with true physiological changes in blood flow.

Herein we show that these challenges can be overcome through scientific advances in coronary vasodilation, technical advances in data acquisition, and integration and analyzation of the results in a computational framework. Specifically, we demonstrate that it becomes possible to accurately detect healthy and myocardium affected by coronary narrowing without contrast agents or ionizing radiation when (a) the heart is repeatedly stimulated with a physiologically tolerable and prospectively targeted increase in arterial CO_2 (PaCO_2); (b) the heart is rapidly imaged with a time-efficient, confounder-corrected whole-heart, free-breathing BOLD-CMR approach; and (c) the resultant BOLD-CMR images are integrated (registered and segmented) and analyzed to arrive at statistical parametric maps. We refer to this approach as cardiac fMRI (abbreviated as cfMRI); the concept is summarized in Fig. 1. We demonstrate the capabilities of cfMRI in a large, clinically relevant animal model with and without coronary stenosis. It is exciting that cfMRI opens the door for accurately imaging myocardial oxygenation. Accordingly, this method can potentially enable noninvasive examination of IHD in millions of people, without exposing them to ionizing radiation or exogenous contrast agents. This is done based on the true parameter determining ischemic heart disease, namely impairments in oxygenation and opens the door to uncovering insights into myocardial oxygenation that were previously not accessible.

RESULTS

Repeat Stimulation for Enabling Robust Myocardial BOLD MRI: Proof-of-Concept Using 2D BOLD-MRI

Previous studies have shown that arterial CO₂ tension when increased by 25 mmHg from baseline levels can accentuate myocardial blood flow by more than 2-fold, a hallmark of potent coronary vasodilators (22); and that such changes can be identified with 2D myocardial BOLD-CMR. However, studies to determine whether repeat exposure of the heart to a predefined CO₂ stimulus can be used to improve the detection of healthy and hypoperfused myocardium with or without coronary stenosis have not been reported. To fill this gap in knowledge, we studied this in two parts. First, we performed experiments in dogs exposed to repeat modulation of arterial CO₂ (normocapnia, end-tidal CO₂ (P_{ET}CO₂) = 35 mmHg; and hypercapnia P_{ET}CO₂ = 60 mmHg) and free-breathing 2D BOLD-CMR to assess whether the repeat stimulations can identify healthy myocardium in dogs (n=5) without coronary stenosis when the resulting BOLD responses are averaged. Next, we performed studies in the same dogs subjected to coronary stenosis (n=5) to identify whether ischemic territories can be identified following repeat modulation of arterial CO₂ (normocapnia, P_{ET}CO₂ = 35 mmHg; and hypercapnia P_{ET}CO₂ = 60 mmHg) and 2D BOLD-CMR on the basis of signal averaging to reduce image noise. We validated our findings against simultaneously acquired ¹³N-ammonia PET.

Typical results from healthy dogs (i.e. without coronary artery stenosis) exposed to intermittent hypercapnia (established with prospective control of PaCO₂; Fig. 2A) in an animal are shown in Fig. 2B. To investigate the dynamic myocardial BOLD response as a function of PaCO₂ in each myocardial segment, we acquired BOLD images of the mid-ventricular myocardium and segmented images according to the American Heart Association (AHA) 6-segment model and measured the BOLD response in each segment (Fig. 2, panel B1). Every segment showed elevated BOLD response during the hypercapnic stimulations that were absent during normocapnia. When this was repeated (i.e. animals were subjected to repeat hypercapnia and normocapnia), the pattern of BOLD response was reproducible across all segments. A map of BOLD response observed following administration of paired PaCO₂ modulation (defined as hypercapnia followed by normocapnia) is shown in Fig. 2, panel B2. The average BOLD response acquired following four repeat stimulation of hypercapnia and normocapnia is shown in Fig. 2, panel B3. Average myocardial BOLD response derived following multiple stimulations was more homogeneous and higher in magnitude compared to a single stimulation. This observation was consistent with myocardial blood flow changes observed with ¹³N-ammonia PET (Fig. 2, panel B4).

To examine whether this approach could be used to improve the identification of myocardial territories subtended by a significant coronary stenosis, we surgically controlled the left-anterior descending coronary artery (LAD) diameter by adjusting the Doppler flow velocity of the vessel as previously described(23). Subsequently we exposed each animal to a single and multiple PaCO₂ stimulations during which time, 2D BOLD-MRI and ¹³N-ammonia PET scans were acquired. BOLD response observed in each segment of the mid-ventricular myocardium (segmented according to the AHA recommendation) was measured. Typical BOLD response that we observed in an animal is shown in Fig. 2, panel C. Myocardial BOLD response to hypercapnia was strong in segments 1 to 5, but not in segment 6 (Fig. 2, panel C1). Maps of BOLD response observed following single PaCO₂ stimulation was relatively heterogeneous (Fig. 2, panel C2), but the

average BOLD response following four repeat stimulations showed a confined region of impaired BOLD response consistent with the LAD territory (Fig. 2; panel C3), which was consistent with ^{13}N -ammonia PET (Fig. 2, panel C4).

Fast, Free-breathing, Whole-Heart Myocardial BOLD-MRI with Repeat Hypercapnic Stimulations for Accurate and Objective Identification of Myocardium Perfused by Healthy Coronary Arteries

While repeat hypercapnic stimulations combined with 2D BOLD-CMR and signal averaging can significantly improve the visualization of standard, single-stimulation, BOLD-CMR, it has practical limitations. First, with this approach the BOLD responses need to be visually delineated which introduces subjectivity into image analysis. Second, 2D CMR acquisition schemes are limited by (a) inadequate speed to fully image the heart multiple times to accommodate repeat hypercapnic stimulations; (b) irrecoverable cardiac motion between multiple acquisitions leading to misregistration errors; and (c) undesirable contributions from T_1 weighing, coil bias, breathing motion and heart rate dependency, all of which confound the BOLD response. Collectively, these limitations can compromise both sensitivity and specificity of BOLD-CMR. We addressed these key limitations in two steps.

In the first step, we performed numerical simulations by considering a range of peak BOLD signal responses and associated noise, to estimate whether the confidence in detecting myocardial BOLD response can be improved by increasing the number of measurements in a statistical test employing repeated measures Analysis of variance (ANOVA). The results (Fig. 3) showed that the statistical confidence ($p < 0.05$) in identifying the presence of a BOLD response is directly related to the maximal dynamic range of the response available and the number of repeat measurements. Specifically, it identified that for a given average myocardial BOLD response in the heart, typically $\sim 10\%$ (23), more than 3 repeat measurements would be needed to objectively identify the healthy myocardial territories. This model provides the basis for developing a statistical framework for objectively discriminating between myocardial regions that are responsive to a given stimulus from those that are not on the basis of repeat measurements.

To address the second limitation, we developed a fast free-breathing 3D T_2 mapping technique at a magnetic field strength of 3T that is insensitive to heart-rate changes between rest and stress states, which would support repeat imaging of the whole heart under multiple hypercapnic/normocapnic stimulations. We then performed in-vivo studies in healthy canines ($n=8$) under repeat hypercapnic/normocapnic stimulations. Subsequently we analyzed the observed BOLD response within an ANOVA framework to derive statistical parametric maps of p-values.

The framework of the data acquisition protocol, image acquisition and reconstruction strategy, and statistical analysis used to analyze the BOLD images is summarized in Fig. 4. The data acquisition protocol under time-varying PaCO_2 (i.e. alternating between normocapnia and hypercapnia) is shown in Fig. 4A. The confounder-corrected T_2 CMR pulse sequence (magnetization preparation, time-efficient k-space sampling, motion-corrected T_2 mapping) that we developed for rapidly imaging the whole-heart under hypercapnic stimulation is shown in Fig. 4B (panels B1-B3). We used the T_2 values to assess the BOLD response. Fig. 4C shows representative whole-heart BOLD response from a dog under a single hypercapnic/normocapnic stimulation using the new imaging sequence. A mid-ventricular BOLD response from the same animal acquired under similar conditions, using the standard short breath-held 2D BOLD-CMR, is shown for reference. The myocardial BOLD response from the 2D and 3D approaches were similar, albeit both approaches showed significant heterogeneity of response. Fig. 4D shows the statistical framework we used to identify the healthy myocardial territories. To achieve this, animals were subjected to repeat hypercapnic/normocapnic stimulations. The whole-heart T_2 images at each state of PaCO_2 acquired under hypercapnic/normocapnic conditions were registered to the

initial 3D myocardial T_2 maps acquired under normocapnia using non-rigid registration (Advanced Normalization Tools, ANTs) (24). Subsequently, animals underwent repeat hypercapnic/normocapnic stimulations. The whole-heart images were segmented according to the recommendation of AHA. Segmental myocardial T_2 values acquired under normocapnia and hypercapnia were compared using all the segments (hypercapnia and normocapnia pair) with ANOVA statistics to test the null hypothesis:

H_0 [Null: BOLD response absent]: T_2 during normocapnia = T_2 during hypercapnia

H_1 [Alternate: BOLD response present]: T_2 during normocapnia \neq T_2 during hypercapnia.

Null hypotheses were rejected when $p < 0.05$. The segmental p -values from repeated measurements one-way ANOVA were used to create statistical parametric maps (SPM) as shown in Fig. 4D. In these maps, myocardial segments with $p < 0.05$ were the segments showing statistically significant BOLD response to hypercapnia following one or more hypercapnic stimulation(s).

Using this approach, we studied healthy dogs exposed to intermittent hypercapnia. A representative case from this study is shown in Fig. 5A, where the segmental p -values were mapped following each stimulation as SPM. Note that, although there is marked heterogeneity in BOLD response following a single stimulation, with each repeat stimulation, the statistical confidence in observing a BOLD response increased and became homogeneous across the heart. A direct comparison between the results in Fig. 5A, averaged across all AHA segments as a function of number of stimulations, is shown along with ^{13}N -ammonia PET map of myocardial perfusion reserve (MPR) in Fig. 5B. The mean and standard-deviation of the p -values observed following each stimulation derived across all myocardial segments of the heart decreased with each repeat stimulation as shown in Fig. 5, panel B1. On the basis of PET images, the MPR derived under hypercapnia and normocapnia in the same animal and imaging session confirmed the absence of perfusion deficits and uniform vasodilatory response across the left ventricle (Fig. 5, panel B2). The collective findings of SPM across all animals, following single and quadruple stimulation blocks, along with mean ^{13}N -Ammonia PET MPR are shown in Fig. 5C (panels C1 and C2), respectively. Following a single stimulation, there is marked heterogeneity in p -values suggesting that healthy myocardium can be mischaracterized to be non-responsive likely due to dominance of noise over small BOLD signal change; however, with repeat stimulation, the noise and thus the errors, are significantly reduced. These results support the notion that when repeat hypercapnic stimulations are combined with confounder-corrected fast 3D BOLD-MRI, it is possible to substantially increase the confidence in detecting healthy myocardial territories without contrast agent or ionizing radiation at levels that are realized with the gold-standard, ^{13}N -ammonia PET.

Accurate Detection of Myocardial Segments Subtended by Clinically Significant Coronary Stenosis with Statistical Parametric Maps Derived Using 3D Myocardial BOLD-MRI and Repeat Hypercapnia

In the previous section, we demonstrated that BOLD response can be accurately detected in healthy myocardium using an approach which integrates the results from a fast 3D technique, repeat hypercapnic stimulation, to arrive at statistical parametric maps. However, whether this approach can be used to identify myocardial territories affected by a functionally significant coronary stenosis is not known. To address this gap and test whether our approach can be extended for identifying reversible perfusion defect territories, we performed additional studies in the same dogs ($n=7$) that underwent 3D acquisitions in the absence of coronary stenosis. Animals were studied with non-flow limiting LAD coronary stenosis

and underwent repeat stimulations (4 blocks; each block consisting of hypercapnia and normocapnia) and whole-heart BOLD images were acquired at each of the hypercapnic and normocapnic states in a similar manner to Fig. 4A. As before, hearts were registered using ANTs(24) and the myocardium was segmented according to the AHA recommendation. The statistical framework was applied using the same hypothesis tests as outlined in the previous section to identify remote (i.e. unaffected/healthy) myocardial territories. The p-values were then used to construct SPMs of the heart.

The findings from this study are summarized in Fig. 6. Panel A, from a representative animal with LAD stenosis shows the myocardial SPM along the long- and short-axis orientations of the heart, along with standard bull's eye representation based on AHA segmentations. Note the significant heterogeneity in BOLD response throughout the myocardium following single stimulation and the convergence of two myocardial territories with increasing number of stimulations. The spatial localization of these territories was visually concordant with the ^{13}N -ammonia PET MPR (Fig. 6B) following repeat stimulation. The segmental territories identified to be remote and affected based on ^{13}N -ammonia PET MPR showed distinct statistical characteristics. For the case in Fig. 6A, mean and standard-deviation of the p-values of all remote territories were significantly lower than the affected territories independent of the number of stimulations. Notably the p-values of the remote territories quickly converged to low values after the second hypercapnic stimulation and reached statistical significance by the fourth hypercapnic stimulation. However, the affected territories retained high p values and were heterogeneous despite the increasing number of stimulations. These observations were consistent with the spatially observed differences in MPR based on ^{13}N -ammonia PET and consistent across all animals (Fig. 6C). We found that regardless of the number of stimulations, nearly all measurements showed a sensitivity that is $>80\%$ in identifying the affected myocardium. However, the specificity for identifying healthy myocardium was only 36% with a single stimulation and with each additional stimulation, the specificity increased significantly, reaching 92% following the fourth stimulation. A similar observation was evident with accuracy: while the accuracy after a single stimulation was 49%, with each increasing stimulation, the accuracy increased substantially and reached 91% following the fourth stimulation. These results support the notion that a statistical parametric mapping, which is enabled by repeatedly stimulating the heart with prospective control of the PaCO_2 and fast, 3D whole-heart T_2 mapping, can markedly increase the accuracy of BOLD-CMR for identifying hypoperfused myocardial territories to levels observed with ^{13}N -ammonia PET.

DISCUSSION

Accurate identification of myocardial territories affected by coronary artery disease is critical for managing patients with ischemic heart disease. Current methods used for this purpose however, require ionizing radiation or exogenous contrast media. In the best case, these methods expose patients to incremental risk; and in the worst case, they are contraindicated. Previous efforts to address these limitations, particularly on the basis of myocardial BOLD-CMR, have made important progress; however, clinical adoption of it remains uncertain due to limited reliability. In this work, we demonstrated how it is possible to overcome this key obstacle through a new approach, which identifies the affected and healthy/remote myocardial territories using a statistical framework. This is enabled by intermittent hypercapnia to repeatedly stimulate myocardial blood flow and rapid, free-breathing whole-heart T_2 mapping to acquire BOLD images, and a computational platform to perform motion-corrected registration and segmentation. Our investigation, performed using a clinically

relevant animal model, systematically demonstrates through a set of progressively advancing studies how this can be accomplished. In the first study, using a 2D T₂ CMR with limited spatial coverage (single, short-axis slice), we show that repeat modulation of myocardial blood flow changes in the heart with hypercapnia allows visual identification of (a) the healthy myocardium in animals without coronary stenosis; and (b) the affected and remote myocardial segments in animals with coronary stenosis. To overcome the spatial coverage and registration limitations inherent to the 2D approach, we developed a time-efficient, confounder-corrected, whole-heart T₂ mapping that can be performed under free-breathing conditions. We then applied this imaging approach with rapid prospective control of PaCO₂ to generate whole heart myocardial BOLD images under hypercapnia and normocapnia. These datasets were then registered together and analyzed segmentally in a statistical framework to demonstrate that SPMs can be generated to accurately identify the healthy myocardium in animals without coronary narrowing. Finally, we extended the approach in animals with controlled coronary artery stenosis so that it can be used to objectively identify healthy and affected myocardium in the setting of clinically significant coronary stenosis with unprecedented sensitivity, specificity and accuracy; all above 90%.

Our study assessed segmental changes in myocardial perfusion based on the changes in myocardial oxygenation associated with clinically significant coronary stenosis. While this is sufficient to meet the current clinical need in the setting of coronary artery disease, expanding this approach to pixel-wise assessment of myocardial oxygenation would open the door for testing novel physiological hypotheses surrounding IHD that are yet to be proven. For instance, pixel-wise cfMRI could be used to evaluate alterations in microcirculatory oxygenation, which could empower the assessment of microvascular disease, where the myocardial blood flow to the subendocardium is believed to be impaired even in the absence of occlusive coronary disease. Current methods do not have the capacity to confirm or refute this since the available methods rely on washout kinetics of the contrast medium and not oxygenation. Hence, pixel-wise assessment of myocardial oxygenation enabled by cfMRI can be instrumental for accurately discerning whether the transmural changes in blood flow and oxygenation occur in parallel. Such an understanding could provide new insights that can improve our understanding of how angina develops in patients with microvascular disease and evaluate therapies to alleviate microvascular impairments in oxygenation. Studies of this nature are likely to demand more advanced segmentation and registration approaches so that pixel-wise analysis can be accurately performed. We anticipate that these demands can be met with novel segmentation and registration algorithms that are actively being developed for cardiac image analysis (25). Additionally, to enable pixel-wise SPM, additional studies would be required to determine the number of minimum stimulations necessary for accurate assessment of BOLD signal changes at the pixel level.

There are multiple other conditions where cfMRI could be useful as well. cfMRI identifies the affected regions of the myocardium as those regions that do not respond to repeat hypercapnic stimulation. Although we used this approach to identify territories affected by stenosis of a single coronary vessel, we anticipate that this approach can be applied to other patterns of coronary artery disease as well. Specifically, this approach may be extended for identifying clinically significant multi-vessel coronary artery disease, which is believed to result in balanced ischemia. In addition, cfMRI may also be used to examine changes in myocardial oxygenation of non-ischemic origin, such as hypertrophic heart disease, which is known to impair myocardial oxygenation reserve (13). Moreover, although our studies showed that cfMRI can identify substantially reduced blood flow and oxygenation, identification of early changes in myocardial oxygenation (e.g., from subclinical level of coronary stenosis or early changes in the heart due to hypertrophy) would require

additional studies. We anticipate that these studies would benefit from refined statistical hypotheses and/or selection of optimal statistical thresholds that build on identifying myocardial territories of interest based on cfMRI.

Finally, given the lack of invasive or non-invasive methods to directly assess myocardial oxygenation in vivo, we demonstrated the capacity of cfMRI to accurately identify myocardial territories affected by coronary stenosis on the basis of ^{13}N -Ammonia PET under identical physiological conditions. Nonetheless, the tapering off in sensitivity and specificity between cfMRI and ^{13}N -Ammonia PET at ~90% can be suggestive of the potential differences between flow and oxygenation. Further studies would be needed to probe the conditions under which myocardial oxygenation and flow changes are congruent or different.

Other methods to assess IHD without contrast agents or ionizing radiation are under development or are emerging (10, 26). Amongst these, spectroscopic CMR approaches have the capacity to offer insight into myocardial oxygenation, but they have not been successfully translated into clinical practice due to poor reliability (27). Additionally, a recent study employing native T_1 CMR has successfully demonstrated that IHD can be identified without exogenous contrast agents or ionizing radiation (10). While this approach appears promising, randomized multi-center studies to evaluate the capacity of native T_1 CMR for diagnosis of IHD in spectrum of patients presenting with the disease is likely needed prior to its widespread adoption.

Practical Aspects of Translating the Proposed Approach into Clinical Setting

The proposed method builds on previous studies from our laboratory and elsewhere, which show that tolerable levels of hypercapnia leads to more than 2-fold increase in myocardial blood flow and similar modulation in oxygenation in extent to that observed with adenosine (commonly used coronary vasodilator) in both in dogs and in humans (22) (28). Although our findings in this study are limited to canines, given that all cardiac stress testing paradigms have been first successfully demonstrated in dogs, and 25-mmHg increase in PaCO_2 is tolerable in humans, we anticipate that the proposed approach would translate well in humans. To date, hypercapnia has been shown to be safe and tolerable in a broad spectrum of patients (age range 9 to 88) (29). Further, hypercapnic stimulus in conjunction with imaging has been extensively studied in patients with neurovascular disease(29). Accordingly, there is precedence for using hypercapnia in patients.

Given the growing availability of MRI systems, the infrastructure costs required to translate the proposed approach into the clinical settings, which already have access to MRI suites, is expected to be minimal (costing less than 1% of the total cost of the scanner environment). Further, since the proposed strategy does not require contrast agents, infusion pumps etc., it would yield substantial cost savings to the medical centers relative to the status quo. Although, a direct translation of the proposed approach in the current state is expected to take ~40 minutes in human subjects, compared to the ~10-15 minutes of imaging duration with standard methods, methods that can reduce scan time (for e.g., through faster data acquisitions taking advantage of spatio-temporal redundancies in conjunction with utilization of generalized linear models for signal analysis), are expected to permit the proposed approach to be executed within the standard duration of cardiac stress tests. This would also limit the hypercapnic durations to a much shorter time (<4 minute).

Although a 25-mmHg increase in PaCO_2 is expected to be tolerable by most people, some may find it uncomfortable, which may be addressed by taking advantage of the flexibility cfMRI offers for fine tuning image acquisition and analysis. For example, in patients who could tolerate only a lower hypercapnic stimulus, based on Fig. 2,

a greater number of weaker hypercapnic stimulations may offer a viable alternative. When this is combined with accelerated data acquisition strategies to yield images of higher temporal resolution to deploy advanced statistical methods that automatically find thresholds of affected regions in a multivariate fashion, it may be possible to identify myocardial territories supplied by stenotic coronary arteries, even in subjects with lower tolerance for hypercapnia (30). Alternatively, in patients who are able to tolerate a 25-mmHg of stimulus but cannot tolerate multiple repeat stimulations of it, an alternative may be rapid acquisition of images under other waveforms of PaCO₂ (e.g. ramps instead of blocks or shorter frequency with longer duration of hypercapnia), which are all possible with the proposed prospective control of PaCO₂.

Although the proposed approach has notable strengths, it is not without limitations. First, while the existing methods utilize pharmacological stress, contrast media and/or ionizing radiation, cfMRI requires a gas controlling system, which is an additional expense and one that may require a skilled operator for gas control, albeit this individual may be no different than one who is typically present during standard pharmacological stress tests, such as a nurse practitioner. Moreover, given that the proposed approach relies on PaCO₂ to alter the vasodilatory capacity of the coronaries, the capability of the approach in subjects with respiratory disorders (asthma, chronic pulmonary disorder, etc.) in whom IHD is suspected is unclear and requires careful investigation. Finally, the proposed approach requires a clinical MRI system with high performance hardware and software, which although is becoming common, it is not always within reach for everyone.

In spite these limitations, the proposed approach opens the door to new opportunities for cardiac stress testing in some of the most vulnerable patients. First, cardiac stress testing may be enabled in adult patients with renal insufficiency, who would otherwise receive multiple doses of ionizing radiation, which can expose them to greater risks associated with radiation. Next, it offers an alternative to patients who are not candidates for exercise or intravenously administered vasodilatory agents as part of stress tests. Further, it may enable cardiac stress testing in the children without ionizing radiation, contrast agents, pharmacological stress or needles. Accordingly, there is substantial motivation to translate the proposed approach not only to incrementally improve existing care but also to enable management of IHD in those who are contraindicated for standard cardiac stress tests.

CONCLUSION

cfMRI enables non-invasive determination of healthy myocardium and myocardium affected by reversible perfusion defects due to coronary stenosis on the basis of myocardial oxygenation with unprecedented reliability. This integrated approach has the capacity to open a new paradigm for a radiation-, contrast- and needle-free approach for accurately determining reversible perfusion defects in patients suspected of having functionally significant coronary artery disease. Further, it has the desirable characteristics to access multiple other myocardial pathologies on the basis of oxygenation.

MATERIAL and METHODS

In the text below we provide an abbreviated version of the Materials and Methods. A more detailed version of the methods including data analysis and statistical tools employed can be found in the Supplementary Material.

Animal Preparation and Method for Inducing Coronary Stenosis

Dogs (N=15, 20-25 kg) were studied with and without surgically induced coronary stenosis. All animals were studied according to the NIH "Guide for the Care and Use of Laboratory Animals" following approval of Institutional Animal Care and Use Committee. In a subset of the animals, a left lateral thoracotomy was performed as previously described by our group (22). A Doppler probe was attached distal to the first branch of the left anterior descending coronary artery (LAD) to enable measurement of coronary blood flow velocity (CBFV). An externally actuated hydraulic occluder was affixed proximal to the Doppler flow probe. Subsequently, the chest was closed, and the animals were allowed to recover for at least 7 days prior to imaging studies. Prior to all imaging studies, animals were fasted, sedated, intubated and anesthetized. During the imaging studies, anesthesia was maintained with a continuous infusion of propofol. Dogs were transferred to the PET/MR scanner table and were mechanically ventilated through the RespirAct™ (Thornhill Research Inc, ON, Canada) with parameters reported in previous studies(23). In stenosis studies, coronary stenosis was induced prior to commencing imaging. The perfusion level during rest and stress were confirmed with $^{13}\text{NH}_3$ PET images. Prior to, or right after, the baseline ($\text{PETCO}_2 = 35$ mmHg) and peak hypercapnia ($\text{PETCO}_2 = 60$ mmHg) BOLD acquisitions, the Doppler transducer (Triton Technology Inc, CA, USA) was connected to the wires originating from the surgically implanted Doppler probe and root-mean-square Doppler flow velocity values were recorded. In dogs where LAD stenosis was to be induced, peak hyperemic coronary blood flow velocity was measured at $\text{PETCO}_2 = 60$ mmHg was reduced to coronary blood flow velocity measured at $\text{PETCO}_2 = 35$ mmHg to provide a standardized hemodynamically effective constriction that does not decrease blood flow below baseline flow under resting conditions (normocapnia).

Modulation of Arterial Pressure of CO_2

Prospective targeting of PaO_2 and PaCO_2 was implemented using a validated gas controlling system (RespirAct™). The principles of controlling end-tidal gases have been previously described (31). In this study, we targeted hypercapnia at $\text{PaCO}_2 = 60$ mmHg with $\text{PaO}_2 = 130$ mmHg; and normocapnia at $\text{PaCO}_2 = 35$ mmHg with $\text{PaO}_2 = 130$ mmHg. These targets were synchronized with cardiac MR (CMR) and PET acquisitions. Before each image acquisition, PaCO_2 level were stabilized at the targeted level for 1 minute to ensure that target PaCO_2 values were reached. Physiologic response to the stimulations is summarized in Table SM-1.

Imaging Protocol

In all imaging studies, ^{13}N -ammonia PET and BOLD CMR images were simultaneously acquired a clinical PET/MR scanner. In animals without coronary stenosis, PET images were acquired under rest and hypercapnia (6 mins) to quantify the MBF under different physiological conditions. A time delay was introduced between sequential PET acquisitions at each physiological condition to ensure sufficient decay of each ^{13}N -ammonia dose (5 half-lives, ~50 minutes). Following the first PET scan, 4 sets of prospectively targeted normocapnia and hypercapnia stimulations were induced using RespirAct™. The PaCO_2 levels were maintained for 5 minutes during each physiological state (Fig. SM-1). BOLD CMR images were acquired 1 minute after reaching the targeted PETCO_2 level. In animals with coronary stenosis, baseline blood flow prior to surgery was compared to baseline flow post-surgery (on the day of stenosis studies) using ^{13}N -ammonia PET. LAD coronary stenoses were induced before the first PET acquisition. Other aspects of the imaging protocol were similar to that implemented in intact animals. A schematic representation of the time course of execution of the study

protocol is shown in Figure SM-1. During repeat stimulations, two BOLD acquisition methods (2D and 3D T2 maps) were used in a subgroup of animals. In 2D studies (N=5 for both intact and stenosis groups), a conventional 2D T2 mapping sequence was prescribed over a mid-ventricular slice. Images were acquired under short breath holds (<10 s) at 2 minutes and 5 minutes after target $P_{ET}CO_2$ values were reached. In the 3D acquisitions, the proposed 3D sequence was prescribed under free breathing conditions starting 1 minute after the targeted $P_{ET}CO_2$ level was reached.

MRI Pulse Sequence Development

A heart-rate independent, free-breathing, 3D T2 mapping prototype sequence with whole-heart LV coverage, which minimizes the sensitivity to B0 and B1 inhomogeneities was developed for the PET/MR system. Adiabatic T2 preparation with spoiled gradient-echo (GRE) readout was used to minimize B0 and B1 artifacts that are otherwise prominent at 3T and confound BOLD signal readouts. To improve imaging efficiency and enable data acquisition under free-breathing conditions, a motion-correction platform with a hybrid Cartesian-radial trajectory was applied that permits near perfect imaging efficiency. To further increase acquisition speed and minimize the signal dependence on heart rate between rest and stress, a Saturation Recovery (SR) preparation was integrated with a constant saturation recovery time (T_{SR}) to reset longitudinal magnetization in every heartbeat (32-34). To minimize any potential confounding effects associated with differences in T1 recovery following T2 preparation under rest and stress (10), data was collected and centrically encoded in the through-plane direction. Images were acquired with 3 incremental T2-preparation times ($TE=0, 24, 55ms$) and T2 maps were reconstructed using a custom-written Matlab (The Mathworks, Natick, Massachusetts) script. The accuracy of the proposed approach were studied with computer simulations and ex-vivo tissue preparations. These and the data CMR acquisition parameters are detailed in Supplementary Material.

Assessment of MBF with simultaneously acquired ^{13}N PET

All PET images were acquired in 3D list mode using ^{13}N -ammonia (100 MBq, IV bolus (30 s) followed by 10 cc saline flush) as the blood flow tracer. Prior to each PET scan, MR images were acquired to correct for photon attenuation. A 2-point Dixon MR imaging pulse sequence was used for segmentation and attenuation correction. PET data was acquired over 10 minutes and was started a few seconds before the ^{13}N -ammonia injection. In animals without coronary stenosis, images were acquired during hypercapnia and at normocapnia to determine the MBF response in the absence of coronary stenosis. In animals with coronary stenosis, images were acquired at rest and during hypercapnia after infliction of LAD stenosis. The MRI attenuation map and PET images were aligned and adjusted by an experienced technologist. Dynamic PET images were reconstructed with different time periods (twelve 10-s, two 30-s, one 1-min, and one 6-min frames, for a total of 10 min). Images were reconstructed with 3 iterations and 3D post filtering with a 5-mm Gaussian kernel. Data were reconstructed with 2-mm pixels for each dynamic frame. Myocardial blood flow (MBF; ml/min/g) were derived from the PET data using the automated QPET software (Cedars-Sinai Medical Center, Los Angeles, CA, USA), as shown previously (35).

List of Supplementary Materials:

Animal Preparation and Method for Inducing Coronary Stenosis

Modulation of Arterial Pressure of CO₂

Imaging Protocol

MRI Pulse Sequence Development

Data Acquisition Parameters

Data Analysis Protocol

Statistical Modeling and Analysis of Myocardial BOLD Response

Figure SM-1. Imaging Study Protocol.

Figure SM-2 Computer Simulations and Ex-vivo Experiments.

Table SM-1: Physiological Parameters During Normocapnia and Hypercapnia

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Competing interests: J.A.F and O.S are part time employees of Thornhill Research Inc and X.B is an employee of Siemens Healthineers.

Data and materials availability: All data associated with this study are present in the paper or supplementary materials.

Figure Captions:

Figure 1: Cardiac fMRI. A schematic showing the three key aspects of cardiac fMRI (scientific and technical advances, and a computational framework) that are employed to enable accurate detection of myocardial oxygenation changes.

Figure 2. Repeat Stimulations and Image Averaging for Enhancing Myocardial BOLD Response. *Prospective Control of PaCO₂ (A1 and A2):* Panel A1 shows the system used for prospectively modulating PaCO₂ and Panel A2 shows the trace of achieved PaCO₂ levels during the scans; *Healthy Animal without Coronary Stenosis (B1-B4):* Panel B1 shows segmental BOLD response in AHA segments 1 through 6 in a healthy animal during the first four blocks of intermittent hypercapnia (4 stimulations), which highlights the dynamic signal response during the repeat stimulations. Panel B2 shows the spatial maps of the BOLD response in the mid-ventricular myocardium after one hypercapnic stimulation and Panel B3 shows the spatial map of the mean BOLD response following 4 hypercapnic stimulations. Panel B4 shows the ¹³N-ammonia PET response (myocardial perfusion reserve) acquired simultaneously with BOLD-MRI. *Animal with Coronary Stenosis (C1-C4):* Panel C1 shows that typical segmental BOLD response across AHA segments 1 through 6 during four blocks of intermittent hypercapnia (4 stimulations) from an animal with significant LAD coronary stenosis. Panel C2 shows the spatial maps of the BOLD response in the mid-ventricular myocardium after one hypercapnic stimulation and Panel C3 shows the spatial map of the mean BOLD response following 4 hypercapnic stimulations. Panel C4 shows the ¹³N-ammonia PET response (myocardial perfusion reserve) acquired simultaneously with BOLD-MRI.

Figure 3. Theoretical Basis for Objective Assessment of Myocardial BOLD Response: Figure shows the relation between BOLD response (vertical axis) and the number of stimulations (horizontal axis) required to establish statistical significance (color-coded p-values). For a given BOLD response, the number stimulations required for reliable assessment ($p < 0.05$) of a change from baseline condition lies at the right of the white dotted line. For example, to reliably detect a BOLD response with peak BOLD signal response of 10%, greater than 3 measurements are needed. The color bar on the right provides the scale for p values associated with statistical significance.

Figure. 4 Cardiac fMRI Framework Integrating MRI, Hypercapnic Stimulation and Statistical Analysis. (A) *Data Acquisition Framework*: The approach used to acquire 3D MRI under periodic changes in PaCO₂ (normocapnic and hypercapnic conditions), preceded by a short-delay (stabilization period) to ensure that the acquisitions are only triggered once the desired PaCO₂ are reached. (B) *Time-efficient, free-breathing, confounder-corrected whole-heart T₂ mapping*: The timing diagram and data encoding strategy are illustrated in Panels B1 and B2, respectively. B1 shows a T₂ preparation scheme composed of composite adiabatic RF pulses and spoiled gradient echo readout are used to minimize B₁ and B₀ artifacts at 3T. A saturation-recovery (SR) preparation was added to eliminate the signal dependence on heart rate between segmented readouts and navigator pulses were added to monitor the respiratory motion during acquisition. B2 shows the centric-encoding scheme with hybrid trajectory to ensure optimal T₂ weighting. B3 shows the motion-correction algorithm and T₂ mapping using a log-transformed linear least-squares fit as previously described used (37). (C) *3D Myocardial BOLD Response*: This panel shows 3D T₂ maps (basal, mid-ventricular, and apical) acquired during normocapnia and hypercapnia (single stimulation block). For reference, results from 2D imaging obtained from a mid-ventricular slice are also shown. BOLD Response (computed as (hypercapnic myocardial T₂ ÷ normocapnia myocardial T₂) x100%) for the 2D and 3D cases are shown on the top panel. (D) *Statistical Framework*: shows a schematic of the statistical framework employing repeated measures one-way ANOVA to discriminate between myocardial segments that are statistically responsive and not, based on the hypothesis testing outlined in text, following each repeat hypercapnic/normocapnic stimulation. The polar maps on the lower row show the AHA segmentation with p-values assigned on the statistical test.

Figure. 5 Application of Cardiac fMRI Approach for Reliable Identification of Healthy Myocardium. (A) *Myocardial Statistical Parametric Mapping (SPM)*: Long- and short-axis volume rendered views of the heart with intensities denoting segmental p-values derived from the statistical framework from a typical healthy animal are shown. The polar maps at the bottom of the panel provide a bull's eye plot of p-values. (B) *Myocardial SPM vs. ¹³N-Ammonia PET in Representative Case*: Panel B1 shows the mean and standard deviation of p-values across all segments for the case in panel (A) as a function of number of stimulation blocks (one through four). Panel B2 shows the corresponding ¹³N-Ammonia PET MPR. (C) *Myocardial SPM vs. ¹³N-Ammonia PET Across all Animals*: Panel C1 shows the average response across all animals and all myocardial segments following one and four stimulations. Panel C2 shows mean and scatter of MPR across all animals in response to hypercapnia.

Fig. 6 Cardiac fMRI Based Statistical Parametric Mapping for Accurate Identification of Myocardial Segments Subtended by Clinically Significant Coronary Stenosis. (A) *Myocardial Statistical Parametric Mapping Under Coronary Stenosis*: Long- and short-axis volume rendered views of the heart with intensities denoting segmental p-values derived from the statistical framework from one dog with clinically significant coronary stenosis is shown. The polar maps at the bottom of the panel provide p-values for the AHA segments. (B) *Myocardial SPM vs. ¹³N-Ammonia PET (for a representative case)*: Left panel shows the mean and standard deviation of p-values across affected and remote segments for the case in panel A as a function of number of stimulation blocks (one through four). Right panel shows the corresponding ¹³N-Ammonia PET MPR. (C) *Myocardial SPM vs. ¹³N-Ammonia PET (for all cases)*: Left panel shows the average response across all animals in the affected and remote myocardial segments following one and four stimulations. Right panel shows the mean and scatter of PET-MPR across all animals in the remote and affected segments following hypercapnia. (D) *Sensitivity, Specificity, and Accuracy*: The results from sensitivity, specificity and accuracy determined following each stimulation (with PET serving as the ground truth) are shown in this panel.